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THE EFFECT OF POLYCHLORINATED BIPHENYLS ON RAT REPRODUCTION

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Abstract—Reproduction, pathology and acute toxicity were studied in Sherman rats exposed to the polychlorinated biphenyls, Aroclor 1254 and Aroclor 1260. Rats exposed to Aroclor 1254 at dietary levels of 20 ppm or more had fewer pups per litter than the controls in the F_{1b} and F_2 generations. The 100 ppm exposure level of Aroclor 1254 increased mortality in the F_{1b} offspring and markedly decreased mating performance of the F_{1b} adults. The 500 ppm dietary level of Aroclor 1260 reduced litter size and decreased survival in the F_1 litters. Dietary levels of 5 ppm Aroclor 1254 and 100 ppm Aroclor 1260 had no effect on reproduction in rats exposed through two generations. Liver weights were increased in 21-day-old F_1 male weanlings at the 1 ppm level of Aroclor 1254 and in either sex of F_1 and F_2 weanlings at 5 ppm or higher levels of both Aroclor 1254 and 1260. Histological changes in the liver and increased liver weights were observed in adult rats exposed to the higher levels. Pregnant rats given Aroclor 1254 at the rate of 100 mg/kg/day on days 7–15 of gestation produced grossly normal litters, but only 30·1% of the pups survived to weaning. Reproduction and pup survival were not affected at dosage rates of 50 mg. Aroclor 1254/kg/day or 100 mg Aroclor 1260/kg/day. Oral LD_{50} values in 3–4-wk-old male rats were 1295 and 1315 mg/kg for Aroclors 1254 and 1260, respectively. The iv LD_{50} for Aroclor 1254 in adult females was 358 mg/kg.

INTRODUCTION

Reproductive defects due to commercial mixtures of polychlorinated biphenyls (PCBs) have been reported in experimental birds (Dahlgren, Linder & Carlson, 1972) and mammals (Ringer, Aulerich & Zabik, 1972). Porphyria (Vos & Koeman, 1970), microsomal-enzyme induction (Street, Urry, Wagstaff & Blau, 1969) and liver damage (Kimbrough, Linder & Gaines, 1972; Miller, 1944) have also been reported in experimental animals exposed to PCB mixtures.

The present investigation was initiated in 1970 to study the effects on reproduction and pathology produced by two American-made PCB mixtures sold under the trade-names Aroclor 1254● and Aroclor 1260●. Aroclor 1254 contains 54% (w/w) chlorine and is composed of 11% tetra-, 49% penta-, 34% hexa- and 6% heptachlorobiphenyls; Aroclor 1260 has 60% (w/w) chlorine, with a composition of 12% penta-, 38% hexa-, 41% septa-, 8% octa- and 1% nonochlorobiphenyls (Thruston, 1971). An almost complete identification of isomers in Aroclor 1254 and Aroclor 1260 has been obtained (Sissons & Welti, 1971). Since 1970, the Monsanto Co., the only US manufacturer of PCBs, has voluntarily reduced sales of Aroclor mixtures and has taken steps to limit their use to closed systems (Monsanto Co., 1971).

The present communication is an account of reproduction studies in rats. Also included are acute toxicity values from preliminary studies and comments on pathology and haematology in animals from the reproduction experiments. A detailed account of the liver pathology of the F_0 rats given 20 ppm and higher dietary levels has been published (Kimbrough *et al.* 1972).

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EXPERIMENTAL

Animals and materials. All animals used in the studies were Sherman-strain rats produced under specific-pathogen-free conditions. Aroclor 1254 (Lot AK-38) and Aroclor 1260 (Lot AK-3) were supplied by Monsanto Industrial Chemicals Company, St. Louis, Mo.

Experimental design and conduct

LD_{50} studies. The single-dose oral LD_{50} was determined for Aroclor 1254 and 1260 in weanling male rats (3–4 wk old). The iv LD_{50} was determined in adult females for Aroclor 1254. Between five and eight groups, each of ten rats, were used to obtain each LD_{50} value. The procedure described by Gaines (1969) was followed for oral dosing; Aroclor 1254 or Aroclor 1260 was dissolved in peanut oil and the solution was administered by oral intubation at the rate of 5 ml/kg. For iv dosing, Aroclor 1254 was first dissolved in peanut oil. One part of this solution was added to nine parts of a 1% lecithin–saline suspension and homogenized. This formulation was administered in a single injection via the tail vein at the rate of 5 ml/kg. A control group of five rats received 10% peanut oil in lecithin–saline in the same volume. Survivors were observed for 10–14 days.

Two-generation reproduction studies. For these studies the rats were fed either untreated ground laboratory chow (control) or ground chow fortified with Aroclor 1254 or Aroclor 1260. The Aroclors were dissolved in ether and mixed with cornstarch. The ether was allowed to evaporate and the Aroclor–cornstarch mixture was mixed into increasing amounts of ground chow. Aroclor 1254 was fed at dietary levels of 0 (control), 1, 5, 20 and 100 ppm, while Aroclor 1260 was fed at levels of 0, 5, 20 and 100 ppm. Because exposure schedules had to be staggered, a specific control group was added each time one or more treated groups were started on a dietary regimen. The F_0 rats were started on the diets at 3–4 wk of age and the F_{1b} rats at weaning. Exposure was continuous through mating, gestation and lactation until the rats were killed. Ten males and 20 females were fed at each dietary level in both the F_0 and F_{1b} generations. The F_0 males at each dietary level and half of the females were caged individually. Other F_0 females and all the F_{1b} rats were caged in groups except during the reproduction cycle. Body weights were recorded weekly on F_0 rats, except during the reproduction phase, and food consumption was measured during wk 2 and 5, the week before each mating and the week after weaning of the F_{1a} litters. F_{1b} rats were weighed when started on the diet, during the week before mating and at sacrifice.

The F_0 rats were pair-mated when 3 and 7 months old to produce the F_{1a} and F_{1b} generations, respectively. Breeding-stock F_{1b} rats were selected at weaning from all available litters and pair-mated when 3 months old to produce the F_{2a} generation. F_{1b} rats on dietary levels of 0, 20 and 100 ppm Aroclor 1254 were mated a second time when 8 months old to produce the F_{2b} generation. Viability counts of offspring were made at birth, day 3, day 7 and day 21 (weaning). Litters were inspected daily for condition and the presence of dead pups. After the F_{1b} offspring had been weaned, ten adult F_0 rats of each sex were killed and their livers were weighed and fixed for histological examination. Following the weaning of the F_{2b} generation, for Aroclor 1254, and the F_{2a} generation, for Aroclor 1260, haematological values (total leucocyte count, haematocrit, haemoglobin and differential leucocyte count) were determined on ten adult F_{1b} rats of each sex at the dietary levels of 0, 20 and 100 ppm. These rats were then killed and weights of the spleen, heart, lungs, brain, kidneys, testes and liver were recorded. Tissues were fixed for microscopic study.

At lower dietary levels, haematology was not done and only the livers were weighed. At dietary levels of 5 ppm or less of both Aroclors, one male and one female weanling from each of ten litters from each generation were killed at 21 days of age and the livers were weighed. F_{2a} weanlings at dietary levels of 0, 20 and 100 ppm Aroclor 1254, and F_{1b} and F_{2a} weanlings at levels of 0, 20 and 100 ppm Aroclor 1260 were killed in the same manner and the livers were fixed for further study.

One-generation reproduction studies. Preliminary studies were conducted on both Aroclors at dietary levels of 0, 100 and 500 ppm. The experimental design was the same as that described for the two-generation studies, except that only ten females were used. This study was terminated after the F_{1b} offspring had been weaned. Rats fed 500 ppm Aroclor 1254 were bred only once. Haematology values and organ weights were determined and tissues were examined microscopically on the F_0 rats.

Post-implantation exposure studies. Stock rats 100 days old were pair-mated. Insemination was verified by microscopic inspection of a daily vaginal smear. Counting the day of insemination as day 0, the females were dosed by oral intubation on days 7–15 of gestation (a total of nine doses). To maintain a constant dose volume on a body-weight basis, Aroclor 1254 or 1260 was dissolved in peanut oil at appropriate concentrations and these formulations were administered at the rate of 5 ml/kg body weight, based on day 7 of pregnancy. Aroclor 1254 was given at dosage levels of 0 (peanut oil only), 10, 50 and 100 mg/kg/day. Aroclor 1260 was given at dosage levels of 0 and 100 mg/kg/day. The females were allowed to deliver and the litters were observed through weaning.

Tissue preparation. Tissues were fixed in buffered 4% formaldehyde solution and stained with haematoxylin and eosin for microscopic examination. An Oil Red O stain on fixed frozen sections was used on occasional livers to demonstrate lipids.

Statistics and calculations

LD_{50} values were calculated by the method of Litchfield & Wilcoxon (1949). A *t* test was used for comparing the litter sizes, organ weights, body weights and haematological parameters of the treated groups with those of the control groups. Survival percentages of offspring were compared by the Mann–Whitney *U* test as proposed by Weil (1970). Food consumption values were arrived at by averaging the consumption of both males and females over the five periods in which food was measured.

RESULTS

LD_{50} studies on Aroclor 1254 and 1260

The single-dose oral LD_{50} for Aroclor 1254 in weanling male rats was 1295 mg/kg with 95% confidence limits of 1136–1476 mg/kg. The lowest lethal dose tested was 1200 mg/kg, and 1000 mg/kg produced mild diarrhoea. Signs of toxicity at lethal levels were diarrhoea, depression and salivation, with death occurring in 1–3 days.

For Aroclor 1260, the single-dose oral LD_{50} in weanling males was 1315 mg/kg with 95% confidence limits of 1174–1473 mg/kg. The lowest lethal dose tested was 1000 mg/kg, 800 mg/kg produced no signs of toxicity. Rats given lethal doses developed diarrhoea and depression, with death occurring in 1–7 days.

Under the same test conditions the estimated oral LD_{50} for Aroclor 1254 and 1260 in adult rats was 4–10 g/kg (Kimbrough *et al.* 1972).

The single-dose iv LD_{50} for Aroclor 1254 in adult female rats was 358 mg/kg with 95%

confidence limits of 328–390 mg/kg. The lowest lethal dose tested was 300 mg/kg; 250 mg/kg produced dyspnoea, depression, diarrhoea and salivation soon after dosing. Most rats died within 25–110 min, but at the highest dose (400 mg/kg), death occurred as soon as 5 min after dosing in some animals. Rats that survived appeared normal after 48 hr. In the control group, which received the formulation vehicle only, one rat developed mild diarrhoea.

Preliminary one-generation reproduction studies on Aroclor 1254 and 1260

The reproduction summary for the Aroclor 1254 feeding studies is presented in Table 1. Exposure for 67 days (at the time of pair-mating) to 500 ppm Aroclor 1254 (37·0 mg/kg/day) resulted in fewer litters, smaller litter sizes and 100% mortality by day 3 in the F_{1a} pups. The survival of offspring of the rats fed 100 ppm (7·2 mg/kg/day) for 67 and 186 days before mating was reduced, only 85·9 and 68·1% of the pups surviving to weaning in the F_{1a} and F_{1b} generations, respectively. Litter sizes from both matings were smaller, but the difference was not statistically significant. The mean body weight of the 100 ppm pups at weaning was 7·8 and 10 g less than that of the controls for the F_{1a} and F_{1b} generations, respectively. This difference was probably dose-related, as the treated litters were smaller, but a statistical evaluation was not done. In general the pups appeared small, but otherwise were normal at weaning.

Aroclor 1260 fed at a dietary level of 500 ppm (35·4 mg/kg/day) for 67 and 186 days prior to mating markedly reduced live-litter size and survival-to-weaning in the F_{1a} and F_{1b} generations (Table 2). No effect on reproduction was observed in rats fed 100 ppm (6·9 mg/kg/day) in either the F_{1a} or F_{1b} generations.

The F_0 rats in the one-generation studies were sacrificed after exposure for 8 months. Organ-weight analysis indicated that the liver was the primary organ affected. As reported in the previous paper, the livers in male rats receiving either Aroclor 1254 or 1260 at dietary levels of 100 and 500 ppm were heavier than in controls; this was also observed in females fed Aroclor 1254 at levels of 100 and 500 ppm or Aroclor 1260 at 500 ppm (Kimbrough *et al.* 1972). The testes-to-body weight ratios of F_0 rats fed Aroclor 1254 or 1260 at the 500 ppm level were greater than those of the controls ($P < 0·025$). The difference in actual weight of the testes, however, was not significant. This observation may reflect the reduced body-weight gain observed in all F_0 rats fed the 500 ppm level of both Aroclors. No difference in other organ weights was found.

After 8 months exposure, terminal haematological values in F_0 rats indicated a reduction in haemoglobin and haematocrit ($P < 0·005$) in both sexes fed 500 ppm Aroclor 1254 or 1260. The haematocrit was reduced in males ($P < 0·05$) on 100 ppm Aroclor 1260, but haemoglobin values were normal in this group. The total leucocyte count was normal in rats fed Aroclor 1260, but there was a shift in the differential count. The number of lymphocytes was increased in females ($P < 0·005$) fed 100 and 500 ppm Aroclor 1260 and in males ($P < 0·05$) fed 500 ppm. A corresponding decrease ($P < 0·05$) in polymorphonuclear leucocytes was observed. Differential values for rats fed Aroclor 1254 were normal, but an increase ($P < 0·001$) in total leucocytes was observed in the 500 ppm females.

Two-generation reproduction studies on Aroclor 1254

F_0 rats fed 100 ppm Aroclor 1254 (7·6 mg/kg/day) for 62 or 188 days before mating produced smaller litters than the controls in both the F_{1a} and F_{1b} generations (Table 1). Survival-to-weaning was not affected in the F_{1a} offspring. Only 73·6% of the 100 ppm F_{1b} pups

Table 1 Reproduction and pups survived in groups of rats fed Aroclor 1254

Table 1. *Reproduction and pup survival in groups of rats fed Aroclor 1254*

Generation	Dietary level (ppm)	Parental exposure (days)†	No. of females mated	No. of litters		Litter sizes‡		Total pups/group				Survival (%)	Mean body weight at weaning (g)
								Born (found)		Alive at			
				Born‡	Weaned	At birth	At weaning	Dead	Alive	Day 3	Weaning		
F_{1a}	0‖	67	10	8	7	11·1	10·6	3	89	86	85	95·5	39·2
	100‖	67	10	9	8	9·4	8·1	6	85	78	73	85·9	31·4
	500‖	67	10	4 (2)	0	4·0*	—	5	8	0	0	0	—
	0	62	20	17	17	12·4	11·8	0	211	205	201	95·3	38·7
	20	62	20	19	19	11·7	11·5	3	222	220	219	98·6	38·3
	100	62	20	19	19	10·7*	10·3	10	204	199	196	96·1	32·9
	0	67	20	18	18	11·2	11·1	2	202	200	200	99·0	38·5
	1	67	20	16	15	9·2	9·1	3	147	145	145	98·6	42·3
	5	67	20	17	17	10·8	10·8	1	184	184	183	99·5	42·1
F_{1b}	0‖	186	10	7	7	11·6	11·6	1	81	81	81	100	37·9
	100‖	186	10	8	6	11·8	8·0	0	94	74	64	68·1**	27·9
	0	188	20	17	17	12·3	11·1	2	209	194	189	90·4	37·1
	20	188	20	18	18	10·4*	10·1	1	188	187	181	96·3	39·0
	100	188	20	20	19	9·5**	7·0	6	189	164	139	73·6	32·1
	0	201	20	18	18	12·3	11·0	6	222	211	198	89·2	40·7
	1	201	20	18	17	11·1	9·8	3	200	194	176	88·0	38·0
	5	201	20	20	19	11·1	10·2	2	222	218	203	91·4	38·9
F_{2a}	0	129	20	18	18	12·5	12·2	0	225	221	220	97·8	35·5
	20	129	20	17	17	10·6*	10·1	5	181	174	171	94·5	36·8
	100	129	20	7 (2)	4	7·2**	5·6	7	36	29	28	77·8**	35·2
	0	125	20	19	19	11·7	11·5	3	223	220	218	97·8	37·4
	1	125	20	15	15	11·7	11·5	0	175	174	173	98·9	37·2
	5	125	19	17	17	12·1	11·7	1	205	199	198	96·6	35·7
F_{2b}	0	274	20	17	16	12·7	11·3	4	216	201	191	88·4	31·9
	20	274	20	12	12	9·6**	8·5	6	115	103	102	88·7	36·1
	100	274	20	4 (2)	2	3·5**	3·5	7	7	7	7	100·0	38·3

† Conception to mating for the parents of the F_{2a} and F_{2b} generations.
‡ Numbers in parentheses indicate numbers of litters in which no live offspring were found.
§ Number of live offspring/live litter born.
‖ One-generation study.
Values marked with asterisks differ significantly from the control value: * $P < 0.05$; ** $P < 0.005$.

survived to weaning, but this was not statistically significant ($P = 0.058$). Because of the poor condition of a number of the 100 ppm F_{1b} pups, all were held for 30-days post-weaning observation. Fifteen of these pups died within 30 days of weaning. The mean body weights of the 100 ppm F_1 generation pups from either mating was about 6 g less than the controls at weaning. This is probably significant, since the treated litters contained fewer pups. A reduction in litter size was also observed in the F_{1b} generation at 20 ppm (1·5 mg kg/day).

F_{1b} rats bred after exposure for 129 or 274 days (conception to mating) to 20 or 100 ppm had smaller litters than the controls (F_{2a} and F_{2b} generations). A marked decrease in mating performance was observed in the 100 ppm group, in which only seven and four litters were born in the F_{2a} and F_{2b} generations, respectively. In each case two litters contained no viable offspring when first observed. The data also suggest a decrease in mating performance at 20 ppm, at which level only 12 F_{2b} litters were produced. Survival-to-weaning was reduced in the 100 ppm F_{2a} offspring. All 100 ppm F_{2b} offspring survived to weaning, but this is of little significance since only seven pups were found alive. No effect on reproduction was observed in rats fed 5 ppm (0·32 mg/kg/day) or 1 ppm (0·06 mg/kg/day) through two generations.

Although reproduction was not affected at lower dietary levels of PCBs, an increase in liver weight in 21-day-old weanlings was found at all levels tested (Table 2). At 5 ppm or higher, the liver-to-body weight ratios of weanlings were increased in both sexes in both

Table 2. *Mean liver weights of 21-day-old rats from parents fed Aroclor 1254 or 1260*

Aroclor	Generation	Dietary level (ppm)	Liver weight			
			Males		Females	
			g	% of body weight	g	% of body weight
1254	F_{1a}	0	1·40	3·69	1·38	3·83
		1	1·81*	4·01*	1·63	3·91
		5	1·92*	4·34*	1·86*	4·43*
	F_{1b}	0	1·58	3·69	1·58	3·84
		1	1·54	3·94*	1·49	3·92
		5	1·77	4·63*	1·72	4·75*
	F_{2a}	0	1·37	3·81	1·28	3·74
		20	2·26*	5·78*	2·23*	6·08*
		100	2·63*	6·97*	2·67*	7·10*
	F_{2b}	0	1·63	3·78	1·42	3·63
		1	1·49	3·74	1·36	3·69
		5	1·56	4·23*	1·38	4·09*
1260	F_{1a}	0	1·54	3·66	1·57	3·92
		5	1·73	4·18*	1·70	4·39*
	F_{1b}	0	1·59	3·73	1·55	3·93
		20	2·06*	5·04*	2·14*	5·18*
		100	2·59*	6·33*	2·59*	6·48*
	F_{1b}	0	1·35	3·59	1·29	3·70
		5	1·58	4·01*	1·52*	4·07*
	F_{2a}	0	1·65	3·82	1·64	4·02
		20	2·06*	5·21*	1·94	5·06*
		100	2·50*	6·12*	2·49*	6·85*
	F_{2a}	0	1·41	3·85	1·36	3·87
		5	1·66*	4·21*	1·60*	4·25*

Values marked with an asterisk differ significantly from the control value: * $P < 0.05$.

the F_1 and F_2 generations, but at 1 ppm the liver enlargement was observed only in the F_{1a} and F_{1b} male weanlings. No liver-weight increase was found in either sex of the 1 ppm F_{2a} weanlings.

In 10-month-old F_{1b} rats, an increase in liver-to-body weight ratios was observed in both sexes at 100 ppm, but at 20 ppm it was seen only in the males (Table 3). At 5 ppm or less no increase in liver weight was found in 6-month-old F_{1b} rats or in F_0 rats exposed for 10 months. Both sexes of F_0 rats fed 20 ppm or more Aroclor 1254 for 8 months had enlarged livers (Kimbrough *et al.* 1972). The testes-to-body weight ratios were increased ($P < 0.001$) in 100 ppm F_{1b} adult males. Body-weight gains of adults in all the test groups were comparable to those of the controls except in the F_{1b} females at 100 ppm, which gained less weight than the controls (Table 3). Reduced haemoglobin ($P < 0.005$) and haematocrit ($P < 0.025$) were observed in female F_{1b} adults at 100 ppm, while F_{1b} males at this level had reduced ($P < 0.05$) haemoglobin only. Haematological values at lower levels were normal. No dose-related signs of toxicity were observed in adult F_0 or F_{1b} rats fed 100 ppm or less.

Two-generation reproduction study on Aroclor 1260

No effect on reproduction was observed in rats fed Aroclor 1260 at levels of 100, 20 or 5 ppm (7·4, 1·5 and 0·39 mg/kg/day) through two generations (Table 4). The liver-to-body weight ratios of 21-day-old pups were increased at all dietary levels (Table 2). This effect was observed at the lowest level tested (5 ppm) in all generations.

An increase in liver-to-body weight ratios was observed at all dietary levels in F_{1b} males between 5 and 7 months old, but only at 100 ppm in the F_{1b} females (Table 3). After 8 months exposure, an increase in liver weight was observed at 20 and 100 ppm in F_0 males but not in females (Kimbrough *et al.* 1972). No effect on liver weight was found in either sex of F_0 rats fed 5 ppm for 8 months. The testes-to-body weight ratios of the 100 ppm F_{1b} males were increased ($P < 0.05$); at 20 ppm the increase was not significant. Body-weight gain in all test groups was comparable to the control groups. Haematological values were normal in the 20 ppm F_0 adults and in F_{1b} adults at 100 ppm or less. No signs of toxicity were observed in any of the test animals.

Post-implantation exposure studies on Aroclor 1254 and 1260

The data on reproduction and survival of offspring from females dosed during organogenesis is summarized in Table 5. Nine oral doses of Aroclor 1254 (100 mg/kg/day) given on days 7–15 of gestation resulted in a decrease in the survival of the pups. At this dose only 30·1% of the offspring of the treated group survived to weaning, compared with 98·2% in the control group, and the mean body weight of the test pups was 7·1 g less than that of the controls at weaning. No effect was observed in the groups receiving 50 mg/kg/day or less.

No effect on reproduction or survival was observed with Aroclor 1260 given at a rate of 100 mg/kg/day.

Summary of pathology after treatment with Aroclor 1254 and 1260

A detailed account of the liver pathology in the F_0 rats fed Aroclor 1254 or 1260 at 20 ppm or higher dietary levels has been published (Kimbrough *et al.* 1972). Of the rats considered in the current report, similar changes were observed in the liver of adult rats, particularly in the F_{1b} generations at the higher dietary levels. The incidence of the various

Table 3. *Body weight, liver weight and liver pathology of F_0 and F_{1b} adult rats fed Aroclor 1254 or 1260*

Generation	Sex	Exposure (days)	Dietary level (ppm)	Terminal body weight (g)	Liver weight		No. of livers examined	Liver pathology (no. of livers affected)						
					g	% of body weight		Enlarged hepatocytes	Inclusions	Foamy cytoplasm	Pigment	Fibrous strands	Adenofibrosis	Nodules
Aroclor 1254														
F_0	M	310	0	601	15·22	2·53	10	0	0	0	0	0	0	0
			1	602	15·88	2·60	10	0	0	0	0	0	0	0
			5	614	15·37	2·51	10	0	0	1	2	0	0	0
	F	313	0	370	10·34	2·80	10	0	0	0	1	0	0	0
			1	358	10·14	2·84	10	1	0	0	4	0	0	0
			5	362	10·66	2·94	10	4	1	0	3	0	0	0
F_{1b}	M	328	0	588	14·51	2·47	10	0	0	0	0	0	0	0
			20	602	16·11*	2·67*	8	8	3	7	1	0	0	1
			100	554	19·43*	3·54*	10	10	5	7	4	2	3	0
	F	328	0	349	9·92	2·85	10	0	0	0	0	0	0	0
			20	340	9·41	2·78	7	7	2	5	7	0	3	7
			100	300*	10·53	3·60*	10†	10	2	8	9	3	5	0
F_{1b}	M	190	0	475	11·13	2·34	10	0	0	0	0	0	0	0
			1	453	11·14	2·44	10	0	0	0	0	1	0	0
			5	495	12·19	2·46	10	2	0	2	0	1	0	0
	F	190	0	287	8·27	2·87	10	0	0	0	0	0	0	0
			1	294	8·44	2·86	10	1	1	0	0	4	0	0
			5	299	8·92	2·98	10	1	0	0	0	1	0	0
Aroclor 1260														
F_0	M	250	0	563	14·45	2·57	10	1	0	0	0	0	0	0
			5	567	15·06	2·65	10	3	0	2	0	0	0	0
	F	250	0	322	10·09	3·13	10	0	0	0	0	0	0	0
			5	331	10·80	3·25	10	1	0	0	0	0	0	0
F_{1b}	M	179	0	472	12·68	2·68	10	0	0	0	0	0	0	0
			20	494	14·72*	2·99*	10	8	5	4	0	0	0	1
			100	509	16·63*	3·27*	10	10	10	3	0	0	0	1
	F	179	0	297	9·66	3·27	10	0	0	0	0	0	0	0
			20	304	10·30	3·40	10	6	0	0	0	0	0	0
			100	304	11·14*	3·70*	10	9	0	1	5	2	1	2
F_{1b}	M	217	0	492	11·97	2·43	10	0	0	0	0	0	0	0
			5	505	13·16	2·60*	10	7	2	2	1	0	0	0
	F	217	0	279	7·93	2·84	10	0	0	0	0	0	0	0
			5	286	8·49	2·96	10	2	0	0	0	0	0	0

† Necrosis in two livers.
Values marked with an asterisk differ significantly from control group: * $P < 0.025$.

	5	286	8·49	2·56	10	2	0	0	0	0	0	0

† Necrosis in two livers.
Values marked with an asterisk differ significantly from control group: $^{*}P < 0.025$.

Table 4. *Reproduction and pup survival in groups of rats fed Aroclor 1260*

Generation	Dietary level (ppm)	Parental exposure (days)†	No. of females mated	No. of litters		Litter size‡		Total pups/group				Survival (%)	Mean body weight at weaning (g)
								Born (found)		Alive at			
				Born	Weaned	At birth	At weaning	Dead	Alive	Day 3	Weaning		
F_{1a}	0§	67	10	9	9	12·3	12·2	2	111	111	110	99·1	37·0
	100§	67	10	8	8	11·6	11·6	0	93	93	93	100·0	3. 6
	500§	67	8	8	3	8·5**	3·3	10	68	55	26	38·2*	36·3
	0	68	20	19	19	11·7	11·2	2	223	217	213	95·5	40·9
	20	68	20	17	17	11·8	11·3	0	201	193	191	95·0	39·8
	100	68	20	17	17	11·6	11·2	1	197	192	191	97·0	38·0
	0	71	20	19	19	11·7	11·4	0	222	218	216	97·3	33·8
	5	71	20	18	18	12·1	11·8	1	217	213	212	97·7	38·5
F_{1b}	0§	186	10	9	8	12·1	10·3	1	109	96	93	85·3	40·9
	100§	186	9	5	5	11·0	10·8	0	55	54	54	98·2	36·2
	500§	186	8	6	2	6·7**	2·3	1	40	38	14	35·0	40·7
	0	187	20	17	17	11·1	11·0	6	189	189	187	98·9	40·8
	20	187	20	16	16	11·9	11·4	1	191	191	183	95·8	41·0
	100	187	20	13	13	10·4	10·1	5	135	131	131	97·0	39·2
	0	188	20	17	16	10·3	9·2	10	175	160	156	89·1	36·1
	5	188	19	16	15	11·2	10·6	2	179	171	170	95·0	39·3
F_{2a}	0	128	20	18	18	10·9	10·3	1	197	187	185	93·9	40·3
	20	128	20	19	19	11·3	11·1	1	215	213	211	98·1	38·7
	100	128	20	20	20	10·1	9·9	1	201	200	197	98·0	38·6
	0	127	20	13	13	11·8	11·6	1	153	152	151	98·7	34·8
	5	127	20	18	18	11·8	11·6	0	213	212	209	98·1	38·5

† Conception to mating for parents of the F_{2a} generation.
‡ Numbers of live offspring/live litter born.
§ One-generation study.
Values marked with asterisks differ significantly from the control value: $^{*}P < 0.025$; $^{**}P < 0.001$.

Table 5. *Reproduction and survival of pups from dams dosed orally with Aroclor 1254 or 1260 on days 7–15 of pregnancy*

Dose (mg/kg/day)	No. of females treated	No. of litters		Litter size†		Total pups/group				Survival (%)	Mean body weight at weaning (g)
						Born (found)		Alive at			
		Born	Weaned	At birth	At weaning	Dead	Alive	Day 3	Weaning		
					Aroclor 1254						
0	9	9	9	12·3	12·1	0	111	110	109	98·2	37·9
100	9	7	5	11·9	3·6	8	83	64	25	30·1*	30·8
0	10	10	10	11·9	11·8	0	119	119	118	99·1	40·6
10	9	9	9	12·1	12·0	0	109	109	108	99·1	37·6
50	10	10	10	13·4	12·7	0	134	131	127	94·8	30·5
					Aroclor 1260						
0	12	12	12	12·0	11·5	4	144	140	138	95·8	33·9
100	12	12	11	11·7	10·3	10	140	135	124	88·6	33·9

† No. of live offspring/litter born.
The value marked with an asterisk differs significantly from the control value: $^*P < 0{\cdot}001$.

changes is given in Table 3. Enlargement of the liver cells, involving primarily an increase of the cytoplasm surrounding the nucleus, was the most consistent finding and was occasionally observed at the 5 and 1 ppm dietary levels. Inclusions within the cytoplasm, which have also been referred to by some authors (Bennett, Drinker & Warren, 1938; Miller, 1944) as hyaline bodies, were observed more frequently in the males than in the females and were only occasionally noticed at the lower dietary levels. At 5 and 1 ppm the cytoplasm of many liver cells was vacuolated or foamy. According to the studies reported previously (Kimbrough *et al.* 1972), these changes were indicative of lipid accumulation. They were more pronounced at the higher dietary levels of 20 and 100 ppm. Adenofibrosis, which was also described in the previous paper, was observed only at the 100 ppm dietary level.

Two additional findings made in adult F_{1b} rats had not been observed previously in the F_0 rats. One was the occasional occurrence of strands of fibrous tissue which traversed the liver. In addition, hepatic nodules measuring up to 0·5 cm in diameter were observed in occasional livers of F_{1b} adults fed 100 ppm Aroclor 1254 or 1260. In addition, of the seven livers studied from F_{1b} adults fed 20 ppm Aroclor 1254, three showed one or more hepatic nodules. The hepatic nodules consisted of enlarged liver cells, which formed well-organized hepatic cords. The cytoplasm of many of these liver cells, which often stained somewhat lighter than the surrounding liver tissue, contained one or more inclusions. The nuclei of the liver cells within the hepatic nodules appeared normal (Fig. 1).

Apart from the findings in the livers, occasional rats of the control as well as the exposed groups showed mild-to-moderate chronic pyelonephritis. The incidence in the exposed group did not differ from that of the controls. One and two female rats fed 20 and 100 ppm Aroclor 1260, respectively, and two male rats fed 100 ppm Aroclor 1254 showed hyperplasia of the thyroid. Hyperplasia of the thyroid was not observed in the controls, but occasionally it does occur spontaneously in our rats. Whether this low incidence of hyperplasia of the thyroid is related to the consumption of Aroclor cannot be established with certainty from this study. No other morphological changes were observed in any of the other organs, including the testes.

The livers of F_{1b} weanlings from the group given the 20 ppm level of Aroclor 1254 showed slight enlargement of the liver cells but no other morphological changes. The livers of ten F_{1b} weanling rats at the 100 ppm level of Aroclor 1254 showed marked enlargement of the liver cells, which had either foamy or vacuolated cytoplasm. At 20 ppm, the livers of ten male and ten female F_{2a} weanling rats showed greatly enlarged liver cells, and the cytoplasm was vacuolated in most instances. Oil Red O stain on a few of these livers showed that vacuolization was due to lipid accumulation. Similar findings were made in four male and four female F_{2a} weanlings at the 100 ppm level of Aroclor 1254.

The livers of ten male and ten female F_{1b} weanling offspring from dams fed 20 ppm Aroclor 1260 had normal livers on microscopic examination, while those at 100 ppm showed enlarged and vacuolated hepatocytes. The livers of ten of 20 F_{2a} weanlings at the 20 ppm level of Aroclor 1260 and 17 of 19 livers of F_{2a} weanlings at the 100 ppm level showed enlarged cells and vacuolated cytoplasm.

At the lower dietary levels of both Aroclor 1254 and 1260 (5 ppm or less), livers of weanling rats were only occasionally examined in the different generations. The only microscopic change observed consisted of slightly enlarged hepatocytes in some of the livers.

DISCUSSION

The results of our studies confirm the capability of some commercial PCB mixtures to alter reproductive processes in mammals. Our data are in general agreement with those of Keplinger, Fancher & Calandra (1971), who observed effects on mating performance and/or pup survival in rats fed 100 ppm Aroclor 1242 or 1254, but found no effect at the same dietary level of Aroclor 1260. In our study, 20 ppm Aroclor 1254 affected litter size and probably decreased mating performance in the second mating of the F_{1b} rats. The trend suggested the possibility that even lower levels might affect reproduction in subsequent generations, but M. L. Keplinger (personal communication 1973) found no effect through three generations in rats fed 10 ppm Aroclor 1254. Reproductive failure in mink exposed to PCBs has also been reported (Ringer *et al.* 1972). Although actual PCB consumption on a mg/kg basis was not reported, the mink is apparently more sensitive to PCB exposure than the rat, since levels of 1 and 5 ppm Aroclor 1254 affected reproduction after only 4 months' exposure. In the present study in rats, fewer pups per litter were observed at dietary levels of 500 ppm Aroclor 1260 and of 20 ppm or more of Aroclor 1254. Reported litter sizes are based on the pups found, but since it is common for the dam to eat dead or defective offspring, the reduced litter size probably reflects decreased fertility and/or increased perinatal and foetal mortality.

Aroclor 1254 (100 mg/kg/day) given to pregnant rats during gestation produced no effect on the viability or morphology of foetuses examined on day 22 of gestation (Villeneuve, Grant, Khera, Clegg, Baer & Phillips, 1971). In the present study under a similar dosing regimen, dams dosed orally during gestation with Aroclor 1254 (100 mg/kg/day) gave birth to litters that were grossly normal, but only 30·1% of the pups survived to weaning. Since PCBs are excreted in milk (Curley, Burse & Grim, 1973; Fries, 1972), it is possible that PCB ingestion via the milk contributed to the increased mortality of pups observed in both the post-implantation and dietary-exposure studies. The milk of rats 11 days after the last of nine daily doses of Aroclor 1254 (50 mg/kg/day) contained 66 ppm of PCB-derived material (Curley *et al.* 1973). Kimbrough *et al.* (1972) estimated the oral LD_{50} for Aroclor 1254 and 1260 at 4–10 g/kg in adult rats. From the oral LD_{50} values in weanlings in the present study (about 1300 mg/kg), it is evident that the Aroclor mixtures tested are considerably more toxic to immature rats than to adults. Although we have no acute toxicity data for PCBs in neonates, nor figures on their milk consumption, it is possible that lacteal exposure could approach a toxic level in suckling rats at the higher exposure levels.

In both the dietary and post-implantation studies, direct PCB exposure occurred *in utero*, since PCBs have been shown to cross the placenta (Curley *et al.* 1973; Grant, Villeneuve, McCully & Phillips, 1971). PCBs have been reported in eggs (Peakall, 1971) and embryonic mortality has been observed in birds treated experimentally with commercial PCB mixtures (Dahlgren *et al.* 1972; Peakall, Linzer & Bloom, 1972). Dahlgren *et al.* (1972) also reported a decrease in survival and a depression of weight gain in 6-wk-old pheasant chicks from hens treated with Aroclor 1254. In the present studies, similar effects were observed in young rats at the higher exposure levels of Aroclor 1254. Besides a reduction in observed litter size, there was additional evidence of late foetal mortality in the group given 100 ppm Aroclor 1254, no live offspring being found in four of the F_2 litters. Thus it is apparent that some PCB mixtures affect the embryo in both mammalian and avian systems, although the mechanism of this effect remains undefined.

Although the suckling rats ate some of the Aroclor-treated diet for 4–5 days before weaning, it is probable that the observed increase in liver weight in 21-day-old rats was

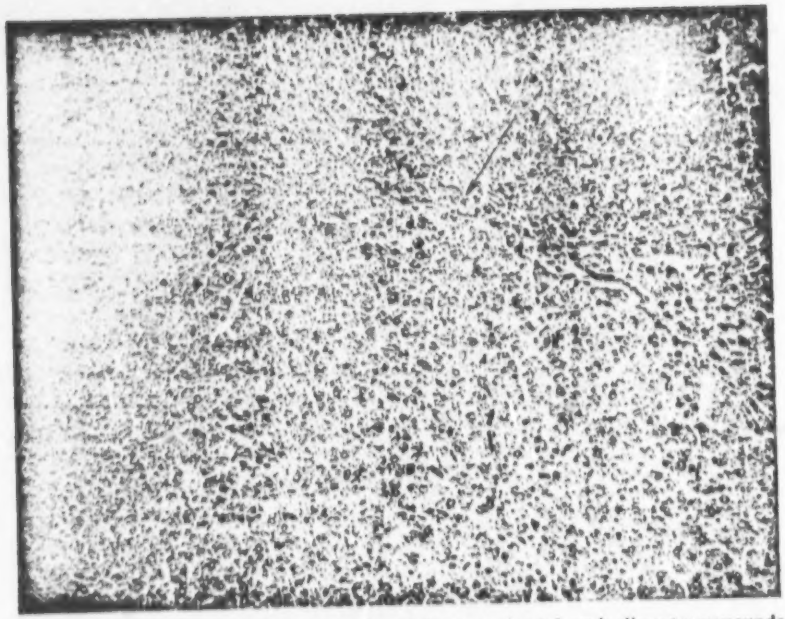

Fig. 1. Section of rat liver, showing a hepatic nodule measuring 0·2 cm in diameter surrounded by normal liver cells (arrow at margin of hepatic nodule). The hepatocytes within the nodule are larger than those of the surrounding tissue and stain less well. Haematoxylin and eosin × 100.

due to PCB exposure via the mothers' milk. Curley *et al.* (1973) also observed a liver-weight increase in weanlings from dams dosed only during gestation. When Aroclor 1254 was given during pregnancy to rabbits, maternal liver weight was increased but not foetal liver weight on day 29 of gestation (Villeneuve *et al.* 1971), thus eliminating the possibility that placental transfer of PCB was the cause of the liver-weight increase seen in weanlings. When adult rats of the F_{1b} generation were killed after dietary exposure for 5–6 months, liver weights at the lower dietary levels were comparable to those in the controls except in males given 5 ppm Aroclor 1260. Presumably once exposure via the milk ceased, the livers returned to normal in spite of a continued low dietary intake of PCB. At a dietary level of 1 ppm Aroclor 1254, only the male weanlings of the F_{1a} and F_{1b} generations had enlarged livers. The absence of this effect in the F_{2a} weanlings is inconsistent. One explanation could be the credibility of the 1 ppm formulation. Problems inherent in consistently reproducing a 1 ppm formulation, as well as contamination of the basal diet with PCBs, are possible sources of error. Over the last 18 months this laboratory has analysed the basal diet for PCB contamination at 6-wk intervals. Most values have been less than 0·1 ppm, but occasionally values of up to 0·5 ppm have been found. The latter level of contamination would introduce a 50% error in a 1 ppm formulation. It is likely that 1 ppm in the parental diet is very near a threshold effect level for the observed liver-weight increase in weanling rats.

At the lower dietary levels, adverse effects were not observed in weanling rats, which exhibited only an increase in liver weight. However, Aroclor 1254 fed to 1-month-old rats at the 100 ppm dietary level (approximately 7·9 mg/kg/day) increased cytochrome *P*-450 and liver weight after 2 days and microsomal protein after 3 days, while a single oral dose of 10 mg/kg increased cytochrome *P*-450 after 24 hr (Goldstein, Hickman & Jue, 1973). A 50–70% increase in nitroreductase activity was observed with Aroclor 1254 and 1260 fed to rats at a dietary level of 0·5 ppm for 4 wk (Litterst, Farber, Baker & Van Loon, 1972). In view of these reports it is very probable that offspring in the reproduction studies, even those involving the lowest levels of PCBs, were subject to inductive effects.

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L'effet des biphényles polychlorurés sur la reproduction du rat

Résumé—On a étudié la reproduction, la pathologie et les indices d'intoxication aiguë chez des rats auxquels on administrait l'un ou l'autre des biphényles polychlorurés Aroclor 1254 et Aroclor 1260. Les rats qui recevaient l'Aroclor 1254 à raison de 20 ppm ou plus du régime avaient des nichées moins nombreuses que les animaux témoins aux générations F_{1b} et F_2. La dose de 100 ppm d'Aroclor 1254 a fait augmenter la mortalité dans la génération F_{1b} et fait nettement diminuer les performances d'accouplement des adultes de cette génération. Administré à raison de 500 ppm du régime, l'Aroclor 1260 a fait diminuer l'importance des nichées et le taux de survie des nichées F_1.

Des taux de 5 ppm d'Aroclor 1254 et de 100 ppm d'Aroclor 1260 sont restés sans effet sur la reproduction des rats soumis à ces régimes pendant deux générations. Le poids du foie a augmenté chez les mâles F_1 âgés de 21 jours au régime à 1 ppm d'Aroclor 1254 et chez les mâles et femelles F_1 et F_2 sevrés des groupes à 5 ppm ou plus d'Aroclor 1254 ou 1260. Des modifications histologiques du foie et des augmentations du poids de cet organe ont été observées chez les rats adultes qui recevaient les plus fortes doses. Des femelles gravides qui avaient reçu 100 mg d'Aroclor 1254/kg de poids vif et par jour du 7ème au 15ème jour de gestation ont mis bas des nichées à peu près normales, mais seulement 30,1% de ces jeunes ont survécu au sevrage. La reproduction et le taux de survie des jeunes n'ont pas été influencés par les doses de 50 mg d'Aroclor 1254 kg jour et de 100 mg d'Aroclor 1260/kg jour. Les valeurs DL_{50} orales chez les rats mâles âgés de 3–4 semaines étaient de 1295 mg/kg d'Aroclor 1254 et de 1315 mg kg d'Aroclor 1260. La LD_{50} intraveineuse d'Aroclor 1254 était de 358 mg/kg chez les femelles adultes.

Der Einfluss von polychlorierten Biphenylen auf die Fortpflanzung der Ratte

Zusammenfassung—Vermehrung, Pathologie und akute Toxizität wurden an Sherman-Ratten untersucht, die den polychlorierten Biphenylen Aroclor 1254 und Aroclor 1260 ausgesetzt wurden. Ratten, die Aroclor 1254 in Konzentrationen von 20 ppm oder darüber im Futter erhielten, hatten weniger Junge je Wurf als die Kontrolltiere in den F_{1b}- und F_2-Generationen. Die Konzentration 100 ppm von Aroclor 1254 erhöhte die Sterblichkeit der F_{1b}-Nachkommen und setzte deutlich die Paarungsleistung der F_{1b}-Erwachsenen herab. Die Konzentration von 500 ppm Aroclor 1260 im Futter verminderte die Grösse der Würfe und verminderte das Überleben in den F_1-Würfen. Die Konzentrationen von 5 ppm Aroclor 1254 und 100 ppm Aroclor 1260 hatten keinen Einfluss auf die Fortpflanzung von Ratten, die ihnen über zwei Generationen ausgesetzt waren. Das Lebergewicht war bei 21 Tage alten männlichen abgesetzten F_1-Tieren bei der Konzentration von 1 ppm Aroclor 1254 und bei beiden Geschlechtern von abgesetzten F_1- und F_2-Jungen bei 5 ppm oder höheren Konzentrationen von Aroclor 1254 und 1260 erhöht. Histologische Veränderungen der Leber und erhöhtes Lebergewicht wurden bei erwachsenen Ratten beobachtet, welche die höheren Konzentrationen erhielten. Trächtige Ratten, die Aroclor 1254 in der Konzentration 100 mg/kg/Tag vom 7. bis 15. Tag der Trächtigkeit erhielten, brachten äusserlich normale Würfe hervor, aber nur 30,1% der Jungen überlebten bis zum Absetzen. Die Fortpflanzung und das Überleben der Nachkommen wurden bei Dosierungen von 50 mg Aroclor 1254/kg/Tag oder 100 mg Aroclor 1260/kg/Tag nicht beeinflusst. Die oralen LD_{50}-Werte bei 3–4 Wochen alten männlichen Ratten betrugen 1295 und 1315 mg/kg bei Aroclor 1254 bzw. 1260. Die iv LD_{50} für Aroclor 1254 bei erwachsenen weiblichen Tieren betrug 358 mg/kg.